IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Adrian K. West, et al.
App. No	:	10/517,653
Filed	:	March 8, 2005
For	:	METALLOTHIONEIN BASED NEURONAL THERAPEUTIC AND THERAPEUTIC METHODS
Examiner	:	Daniel E. Kolker
Art Unit	:	1649
Conf #	:	5626

DECLARATION UNDER 37 CFR § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, Adrian Keith West, a named inventor of the above-captioned application, declare and state as follows:

1. I received my Ph.D. degree in molecular biology from the University of Melbourne in 1987.

2. Currently, I am a senior member of staff at the Menzies Research Institute at the University of Tasmania. I have been at this position and related positions since 1987. I have been Associate Head, School of Medicine, University of Tasmania since 2007.

3. I am a co-inventor of the subject matter claimed in the above-captioned application. I have read and understand the specification of United States Patent Application No. 10/517,653. Additionally, I am familiar with the prosecution history of this patent application, including the subject matter of the currently pending claims.

4. I understand from the outstanding Office Action dated February 20, 2009 that the Examiner rejected certain pending claims of the present application as being anticipated

by and/or as being obvious over Penkowa *et al.* (2002, Journal of Comparative Neurobiology 444(2): 174-189) alone or in combination with other references. In addition, the Examiner rejected some pending claims as being anticipated by and/or as being obvious over Giralt *et al.* (2002, Experimental Neurology 173: 114-128).

5. Giralt and Penkowa state that after an intraperitoneal injection of 5μg/10g body weight, Zn-MT-2 (Giralt et al, 2002), or 5.83 μg per mouse (ie, approximately 2.5 μg/10g body weight, Penkowa et al, 2002), is "readily observed in the extracellular space 30-45 minutes after its injection, decreasing thereafter" (Giralt et al, 2002). They performed these experiments in mice which concurrently received a specific type of brain injury (cryolesion).

6. No data was shown in either paper by Giralt and Penkowa. Nonetheless, these papers were suggested by the Examiner to establish the concept that an intraperitoneal administration of metallothionein could lead to the presence of metallothionein in the brain after injury, and that the administered metallothionein could produce effects including i) the effects Giralt and Penkowa observed, and ii) the different and separate effects as cited in the instant application, for example, that metallothionein acts directly on injured neurites to promote outgrowth.

7. In the present application, Applicants claim, among other things, a method that can deliver a sufficient amount of metallothionein to a target living neuron or live neruonal area to stimulate outgrowth of neurites. As noted above, Giralt and Penkowa neither provided data of the presence of administered metallothionein in the target tissue nor the actual quantity of metallothionein that reached the tissue. Therefore, I and my colleagues tested whether metallothionein administered intraperitoneally via methods of Giralt and Penkowa can be observed in the injured brain and more importantly whether a sufficient amount of metallothionein required to promote neuronal regeneration reaches the injured brain. In this experiment, done with mice, the 1-10 μg/ml of metallothionein should be delivered to the brain to promote neuronal regeneration as shown in the Examples 1 and 2, Figures 1- 10 of the present application.

8. I and my colleagues administered metallothionein by the same route and also performed the same cryolesion as described in the references. We chose a time-point midway in the period mentioned by Giralt and Penkowa (40 minutes). We made two

important modifications. The first modification is that we performed the experiment in transgenic mice which lacked their own (mouse) metallothionein genes (MT-1 and MT-2). Therefore any metallothionein detected in the brains of the experimental animals must be due to administered metallothionein, and cannot be confounded by endogenous mouse metallothionein. The second modification is that we increased the dose of metallothionein 50 fold (to 250 µg/10 g body weight, intraperitoneal) to bias the experiment heavily towards the reported Giralt and Penkowa outcome.

9. We searched for the presence of metallothionein by two methods.

Immunohistochemistry

Firstly, using immunohistochemistry we attempted to detect metallothionein in the vicinity of the cryolesion. This is a qualitative method which detects metallothionein directly in the tissue and gives information about its location (inside cells, outside cells, etc). This was the method used by Giralt and Penkowa in their publications. The experimental results including two microscopic images are attached as Exhibit A. As readily seen in the attached images, we were unable to detect metallothionein by the method of Giralt and Penkowa, in any site within or around the cryolesion, or in any other region of the mouse brain (Fig 1A). This included intracellular and extracellular sites, and also around blood vessels. We used an identical approach to positively detect metallothionein in transgenic mouse kidney (Fig 1B), demonstrating the validity of this technique.

Western Blotting

Secondly, we used western blotting. In this technique, the tissue containing the lesion was removed, dissociated and prepared so that its proteins can be separated by gel electrophoresis. Specific proteins are then visualized by antibody detection. This method is quantitative, highly sensitive and unambiguous, and we are skilled in its use to detect metallothionein. The experimental result is attached as Exhibit B. Positive controls on the gel indicated the technique worked as intended, however we were unable to detect metallothionein in the vicinity of the cryolesion by this method. *See* Figure 2 in Exhibit B. Furthermore, by the use of standard amounts of metallothionein as a comparison, we are able to validate that this experiment would have been able to detect metallothionein in the µg/g range in the brain samples, had it been present.

10. The above-presented experimental data indicate that administration of metallothionein, even in a 50 fold higher dose than as originally stated in Giralt and Penkowa, cannot be detected in the vicinity of a cryolesion under the conditions described by the references. This proves that the methods taught by Giralt and Penkowa cannot deliver a sufficient amount of metallothionein required to promote neuronal regeneration to a target tissue as claimed in the instant invention. Therefore, the subject matter of the currently pending claims is neither anticipated by nor obvious over the cited references.

11. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements and the like so made are punishable by fine or imprisonment, or both, under Section 101 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Dated: 18.08.09
(August)

Adrian Keith West, Ph.D.

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